PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 31/00 | A2

(11) International Publication Number: **WO 00/56297**

(43) International Publication Date: 28 September 2000 (28.09.00)

(21) International Application Number: PCT/IB00/00393

(22) International Filing Date: 21 March 2000 (21.03.00)

(30) Priority Data:
9906715.9 23 March 1999 (23.03.99) GB

(71) Applicant *(for all designated States except US)*: FERRING B.V. [NL/NL]; Polaris Avenue 144, NL–2132 JX Hoofddorp (NL).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: BROQUA, Pierre [FR/FR]; 18, rue des Bergeronnettes, F–01710 Thoiry (FR).

(74) Agent: GEERING, Keith, Edwin; Reddie & Grose, 16 Theobalds Road, London WC1X 8PL (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: COMPOSITIONS FOR PROMOTING GROWTH

(57) Abstract

Inhibitors of dipeptidyl peptidase IV and pharmaceutical compositions comprising these inhibitors are useful in the treatment of short stature due to Growth Hormone deficiency and for promoting GH–dependent tissue growth or regrowth.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

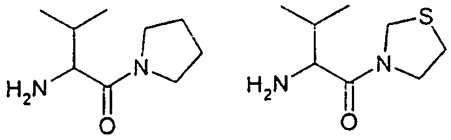
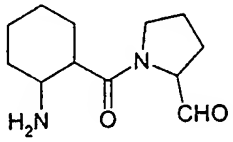
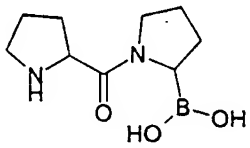
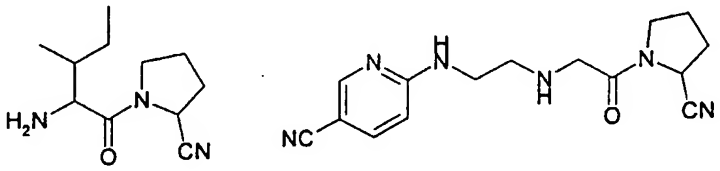
Compositions for Promoting Growth

The present invention relates to agents and compositions for promoting animal growth, e.g. in underdeveloped humans, and for accelerating tissue repair and regeneration.

Inhibitors of DP-IV

Dipeptidyl peptidase IV (DP-IV, also dipeptidyl aminopeptidase IV, DPP-IV, DAP-IV, EC 3.4.14.5) is a serine peptidase that cleaves the amino-terminal dipeptide from peptides and proteins. It recognises substrates wherein the N-terminal sequence is X-Pro or X-Ala. Inhibitors of DP-IV have been proposed as therapeutic agents for the treatment of inflammatory diseases and AIDS. Generally, the known inhibitors of DP-IV are analogues of the substrate. Examples of DP-IV inhibitors are those disclosed in DD 296 075 A5 (Neubert *et al.*, November 1991), WO91/16339 (Bachovchin *et al.*, October 1991), WO93/08259 (Bachovchin *et al.*, April 1993), WO95/15309 (Jenkins *et al.*, June 1995), WO98/19998 (Villhauer, May 1998), WO99/46272 (Scharpe *et al.*, September 1999) and WO99/61431 (Demuth *et al.*, December 1999). Prodrugs of some of these inhibitors have also been described in WO99/67278 and WO99/67279 (both Demuth *et al.*, December 1999).

The following table sets out general types of DP-IV inhibitor compounds, and specific examples thereof which are amongst those preferred for use in the present invention; it also indicates the patent publications from whose broader range of disclosed compounds these types and examples are drawn. It is emphasised that all DP-IV inhibitors disclosed in the quoted DD and WO specifications can be used in the present invention, and reference is positively directed to these prior specifications for full information on the general and more specific formulae and individual compounds concerned. For example, in the table below the indicated pyrrolidine and thiazolidine rings can be replaced by a wide range of other heterocycles of various ring sizes and/or the indicated aminoacyl moieties can be replaced by a wide range of others, as taught by the indicated publications, to give other DP-IV inhibitors for use in the present invention.

Amino-acyl pyrrolidides and thiazolidides (see DD 296 075 A5), e.g. 
Amino-acyl pyrrolidine aldehydes (see DD 296 075 A5 and WO95/15309), e.g. 
Amino-acyl pyrrolidine boronic acids (see WO91/16339 and WO93/08259), e.g. 
Amino-acyl pyrrolidine nitriles (see WO95/15309 and WO98/19998), e.g. 

Actions of Growth Hormone

Growth Hormone (GH, Somatotropin) is secreted by the pituitary in response to a hypothalamic signal in the form of Growth Hormone Releasing Hormone (GHRH). The most abundant isoform of human GH is a 191-residue peptide and GHRH is a 44-residue peptide. GH is a key factor in promoting the normal development of children, and GH deficiency can lead to dwarfism. In adults the importance of GH is less well defined, although plasma levels of GH are similar in adults and children. It is possible that GH plays a role in tissue repair following injury.

The current therapeutic options for GH deficiency include the administration of synthetic GH or of GHRH. However, since both these hormones are peptidic, it is not possible to give them orally. Administration is usually by injection, which can be distressing for the patient (and particularly for children) despite the development of needleless injection systems.

Furthermore, a single injection of GHRH is only an approximation of the pulsatile release of this factor from the hypothalamus. Finally, because both are relatively large peptides. both GHRH and particularly GH are expensive to produce. In consequence, there exists an unmet need for a therapeutic agent that will promote the actions of GH. We have now found that DP-IV inhibitors can increase the concentration of circulating GH.

A first aspect of the present invention is a pharmaceutical composition for the treatment of dwarfism and sub-normal growth (e.g. in children), and for promoting tissue repair (e.g. following injury), which composition is characterized by the inclusion of inhibitor of DP-IV. A second aspect of the present invention is a novel use of inhibitors of DP-IV, namely in the treatment of dwarfism and tissue injury. A third aspect of the invention is an improved protocol for accelerating the growth of children and the regeneration of injured tissue, wherein the subject is administered a composition comprising DP-IV inhibitor.

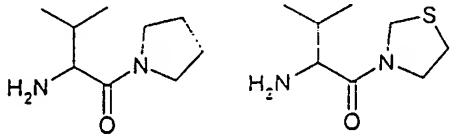
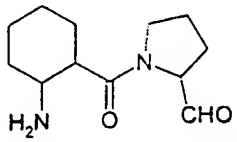
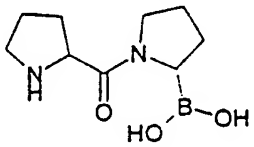
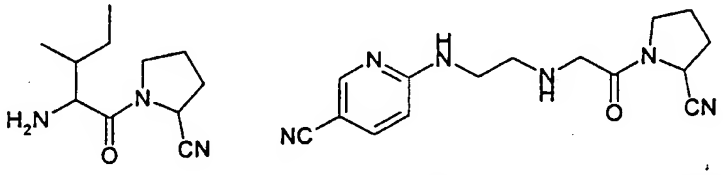
The use of DP-IV inhibitors in this way presents many advantages over current treatment regimens that involve GH or GHRH. GHRH and particularly GH are large peptides that are either isolated from natural sources (such as animal brain preparations) or prepared in culture using recombinant cells. Isolation from animal sources requires that attention be paid to risk of disease transmission and the presence of antigenic protein contaminants. Recombinant hormones are less likely to transmit human pathogens but are still potentially contaminated with antigenic protein. GHRH can be prepared synthetically, but such a synthesis is expensive and necessitates careful purification of the product. In contrast, DP-IV inhibitors are small molecules that are readily accessible using standard synthetic methods. They are non-antigenic, easy to purify and inexpensive.

A further advantage is that DP-IV inhibitors are in many cases biologically active after oral administration. This is in contrast to GH and GHRH, which must be administered by injection. Hence the use of DP-IV inhibitors leads to a less invasive protocol that is less stressful for the patient.

In a first aspect, the present invention provides a pharmaceutical composition for the treatment of sub-normal development or dwarfism. The composition is particularly effective for the treatment of sub-normal development or dwarfism due to growth hormone deficiency. The invention equally provides a composition for the promotion of tissue regeneration.

These compositions are characterized in that they comprise inhibitor of DP-IV. The compositions may further include such pharmaceutically acceptable excipients as are generally known in the art, such as diluents, carriers, bulking agents, binding agents, dispersants, stabilizers and the like.

In the context of the present invention, a compound is considered to be an inhibitor of DP-IV if it inhibits the action of the enzyme at a concentration of 1μM. Preferably, such a compound inhibits the action of DP-IV at concentrations below 100nM and does not inhibit other enzymes at concentrations below 1μM. The following table sets out general types of DP-IV inhibitor compounds, and specific examples thereof which are amongst those preferred for use in the present invention; it also indicates the patent publications from whose broader range of disclosed compounds these types and examples are drawn. It is emphasised that all DP-IV inhibitors disclosed in the quoted DD and WO specifications can be used in the present invention, and reference is positively directed to these prior specifications for full information on the general and more specific formulae and individual compounds concerned. For example, in the table below the indicated pyrrolidine and thiazolidine rings can be replaced by a wide range of other heterocycles of various ring sizes and/or the indicated aminoacyl moieties can be replaced by a wide range of others, as taught by the indicated publications, to give other DP-IV inhibitors for use in the present invention.

Amino-acyl pyrrolidides and thiazolidides (see DD 296 075 A5), e.g. 
Amino-acyl pyrrolidine aldehydes (see DD 296 075 A5 and WO95/15309), e.g. 
Amino-acyl pyrrolidine boronic acids (see WO91/16339 and WO93/08259), e.g. 
Amino-acyl pyrrolidine nitriles (see WO95/15309 and WO98/19998), e.g. 

In a preferred embodiment of the invention, the inhibitor of DP-IV is an amino-acylpyrrolidine nitrile. Particularly preferred are those amino-acyl pyrrolidine nitriles disclosed in WO95/15309 and WO98/19998.

The compositions according to the present invention may be formulated for administration to human subjects by any of the known routes, including oral administration, transmucosal administration (such as buccal, sublingual, intranasal, vaginal and rectal administration), transdermal administration or injection (including intravenous, intramuscular and subcutaneous injection). A preferred route of administration is oral administration. In this case the composition is suitably formulated as a tablet or capsule.

In a second aspect, the present invention comprises a new use for compounds that are known to be inhibitors of DP-IV, which is as therapeutic agents for the treatment of sub-normal development and dwarfism or for the promotion of tissue regeneration.

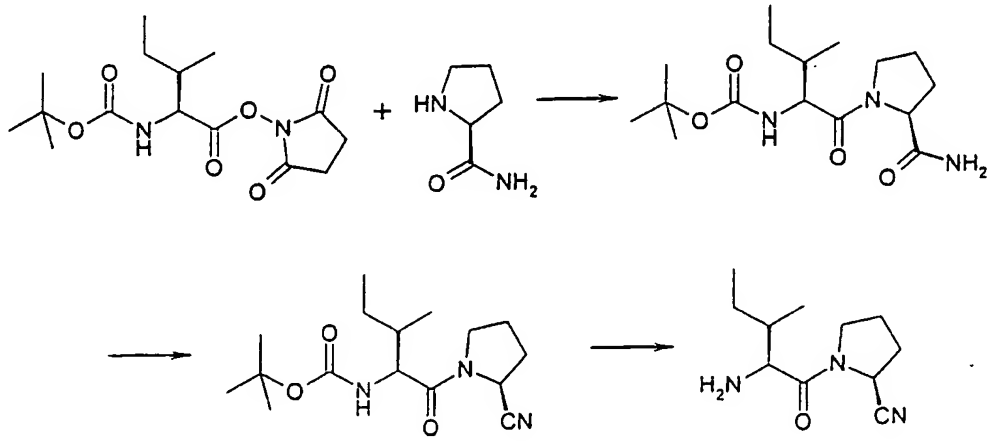
In a third aspect, the present invention comprises an improved method for the treatment of sub-normal development and dwarfism, particularly that due to growth hormone deficiency, and for the promotion of tissue regeneration, wherein the patient is administered a pharmaceutical composition comprising a therapeutically effective amount of inhibitor of DP-IV. The treatment may involve the use of said composition alone or in conjunction with other agents such as have been described heretofore. The administration may be as a single dose or as divided doses taken at intervals of, for example, 2 - 6 hours. In a particularly preferred dosing schedule, the composition is given once per day in the evening prior to sleep. The course of treatment might last for a period of a few days or weeks when used to promote tissue regeneration, or for a longer period when used to treat sub-normal development. The responsible physician will determine when a suitable clinical endpoint has been reached, as well as the details of the dosing regimen.

Examples

Example 1. Preparation of Inhibitors

The inhibitors of DP-IV can be prepared following the methods outlined on the literature. The synthesis of amino-acyl pyrrolidine nitriles is described in WO95/15309 and WO98/19998. The following method is illustrative of these methods.

Example 1A - Synthesis of (2S)-N-isoleucylpyrrolidine-2-carbonitrile.



(a) tert-*Butyloxycarbonyl-isoleucylprolinamide*

To a stirred suspension of prolinamide hydrochloride (225mg, 1.50mmol) in dry dichloromethane (15mL) was added diisopropylethylamine to give a clear basic (pH 9) solution. N-(*tert*-Butyloxycarbonyl-isoleucyloxy)succinimide (328mg, 1.0mmol) was added in one portion and the mixture was stirred at room temperature for 16 hours under a nitrogen atmosphere. The solvent was evaporated *in vacuo* and the residue was partitioned between ethyl acetate and 0.3*N* potassium hydrogensulphate solution. The organic layer was washed with saturated sodium hydrogencarbonate solution, water and brine, dried over sodium sulphate, and concentrated *in vacuo*. The residue was purified by filtration through a short plug of silica gel, eluting with hexane/ethyl acetate (10:90) then ethyl acetate. Concentration of the product-containing eluate gave the title compound as a colourless foaming glass; 301mg (92%).

^{1}H NMR ($CDCl_3$): δ 6.90 (1H, br. s); 5.51 (1H, br. s); 5.18 (1H, d, J=9.6Hz); 4.62 (1H, dd, J=2.6 & 7.0Hz); 4.29 (1H, dd, J=8.4 & 9.2Hz); 3.79-3.58 (2H, m); 2.36 (1H, m); 2.09-1.57 (5H, m); 1.43 (9H, s); 1.17 (1H, m); 0.95 (3H, d, J=6.6Hz); 0.90 (3H, t, J=7.3Hz) ppm.

(b) *(2S)*-N-*(*tert-*Butyloxycarbonyl-isoleucyl)pyrrolidine-2-carbonitrile*

To a stirred solution of the amide of part (a) (203mg, 0.62mmol) in dry pyridine (10mL) under a nitrogen atmosphere was added imidazole (84mg, 1.24mmol). The mixture was cooled to -35°C and then phosphorus oxychloride (0.25mL, 2.48mmol) was added dropwise. The mixture was stirred for 1 hour, during which time the temperature was allowed to rise to -20°C, and the solvent was evaporated *in vacuo*. The residue was purified by chromatography on silica gel to give the title compound as a colourless oil; yield 180mg (94%).

^{1}H NMR ($CDCl_3$): δ 5.14 (1H, d, J=9.2Hz); 4.80 (1H, dd, J=2.6 & 7.1Hz); 4.22 (1H, dd, J=7.9 & 9.1Hz); 3.81 (1H, m); 3.71 (1H, m); 2.30-2.12 (4H, m); 1.75 (1H, m); 1.60 (1H, m); 1.42 (9H, s); 1.19 (1H, m); 0.97 (3H, d, J=6.9Hz); 0.91 (3H, t, J=7.3Hz) ppm.

^{13}C NMR ($CDCl_3$): δ 171.7; 155.6; 118.0; 79.6; 56.0; 46.5; 46.0; 37.8; 29.6; 28.1; 25.0; 24.2; 15.2; 10.9 ppm.

(c) *(2S)-N-(Isoleucyl)-pyrrolidine-2-carbonitrile trifluoroacetate*

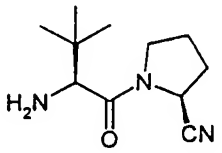
The nitrile of part (b) was dissolved in trifluoroacetic acid and the solution was stirred at room temperature for 1 hour. The solvent was evaporated *in vacuo* and the residue was dissolved in water. The solution was lyophilised to give the title compound as a white fluffy solid; yield 60mg.

FAB Mass Spec.: Calculated m/e 209.3; Found 210.2 $(M+H)^+$

^1H NMR (D_2O): δ 4.3 (1H, m); 3.64 (1H, d, J=5.6Hz); 3.16 (2H, m); 1.86-1.48 (5H, m); 0.98 (1H, m); 0.68 (1H, m); 0.51 (3H, d, J=6.9Hz); 0.38 (3H, t, J=7.3Hz) ppm.

^{13}C NMR (D_2O): δ 169.7; 119.7; 57.3; 48.6; 48.1; 36.9; 30.2; 25.8; 24.5; 15.4; 11.5 ppm.

Example 1B - Synthesis of (2S)-N-((2'S)-2'-amino-3',3'-dimethylbutanoyl)pyrrolidine-2-carbonitrile.



This was prepared following the method of Example 1A by replacing the isoleucine derivative with the corresponding *tert*-butylglycine derivative.

^1H NMR (CD_3OD): δ 4.86-4.81(1H, m); 4.04(1H, s); 3.77-3.71(2H, m); 3.34(2H, s); 2.34-2.08(4H, m); 1.14(9H, s) ppm.

^{13}C NMR (CD_3OD): δ 167.40, 117.99, 58.78, 46.53, 34.21, 29.54, 25.22, 25.03 ppm.

Example 2. Effect on circulating Growth Hormone

Animals

Experiments were conducted in male Sprague-Dawley rats (200-220g) obtained from Iffa Credo (L'Arbresle, France), fed with standard laboratory chow *ad libitum* and kept on a 12 h light-dark in a temperature- and humidity-controlled room.

Surgery

Rats were weighed and anesthetized with Narcorene. The ventral side of the throat was shaved and an incision was made down the center of the throat. The right jugular vein was exposed and cannulated using a polythene tubing (OD 1.0mm) connected to a medical grade silicone tubing (OD 0.94mm) (silicone side in the jugular vein). The tubing was secured and the polythene side was externalized through an incision made on the dorsal side of the neck. The catheter was rinsed with 300µL of a Ringer solution containing 0.1% heparin. Rats were allowed to recover for at least 24 hours in individual cages with food and water available *ad libitum.*

Experimental procedure

The compound of Example 1B (3mg/kg, i.v.) or its vehicle was injected 15 minutes before an injection of GHRH (0.1µg/kg, i.v.). Blood samples (200-250µL) were removed immediately before inhibitor and GHRH injections, and then 5, 10, 15, 20, 30 and 60min post-GHRH. Each blood sample was replaced with an equivalent volume of a Ringer solution containing 0.1% heparin. Plasma was extracted and stored at –20°C until determinations of GH by RIA.

Results

The compound of Example 1B potentiated the GH surge induced by the administration of GHRH (Table 1). Integrated GH responses were 720.2 ± 217.0 ng/ml.60 min and 5072.8 ± 837.3 ng/ml.60 min in the vehicle- and inhibitor-treated group respectively ($p < 0.05$).

Table 1: Effects of the DP-IV inhibitor of Example 1B on the GH surge induced by an intravenous injection of GHRH. Results are mean GH ng/ml of 6 rats ± s.e.m.

	Time (min)								
	-15	0	5	10	15	20	30	60	AUC
GHRH	42.1 ±12.5	40.1 ±8.0	155.7 ±25.1	91.0 ±15.6	61.0 ±12.9	53.6 ±12.5	34.1 ±8.2	26.7 ±3.0	720.2 ±217.0
GHRH + Inhibitor	50.5 ±16.4	33.4 ±6.9	483.0 ±91.0	309.5 ±55.40	164.3 ±28.3	103.1 ±18.1	52.1 ±7.7	26.3 ±3.3	5072.8 ±837.3

The results obtained indicate that inhibitors of DP-IV are able to increase circulating levels of growth hormone and hence are useful in the treatment of sub-normal development due to growth hormone deficiency and other conditions where the promotion of tissue growth is important.

Example 3. Pharmaceutical formulation

3A – 50mg Tablet

Tablets containing the equivalent of 50mg of the compound of Example 1A as the active agent are prepared from the following:

Compound of Example 1A (as trifluoroacetate salt)	154.5g
Corn starch	53.5g
Hydroxypropylcellulose	13.5g
Carboxymethylcellulose calcium	11.0g
Magnesium stearate	2.0g
Lactose	165.5g
Total	*400.0g*

The materials are blended and then pressed to give 2000 tablets of 200mg, each containing the equivalent of 50mg of the free base of the compound of Example 1A.

3B – 100mg Vaginal suppository

Suppositories suitable for vaginal administration and containing the equivalent of 100mg of the compound of Example 1A as the active agent are prepared from the following:

Compound of Example 1A (as trifluoroacetate salt)	154.5g
Corn starch	210.0g
Colloidal silica	2.5g
Povidone 30	49.0g
Magnesium stearate	23.0g
Adipic acid	57.0g
Sodium bicarbonate	43.0g
Sodium lauryl sulphate	5.0g
Lactose	456.0g
Total	*1000.0g*

The materials are blended and then pressed to give 1000 suppositories of 1g, each containing the equivalent of 100mg of the free base of the compound of Example 1A.

The foregoing Examples are illustrative of the invention as disclosed herein, but are not intended to be limiting. Such extensions as would be considered equivalent by one skilled in the art are included within the scope of the invention and the Claims that further define that scope.

One or more DP-IV inhibitors may be used as the sole component active for the specified purposes of the composition and method of the invention.

Claims

1. A pharmaceutical composition for the treatment of sub-normal growth or dwarfism, which composition is characterized in that it comprises a therapeutically effective amount of inhibitor of dipeptidyl peptidase IV.

2. A composition according to Claim 1, wherein the sub-normal growth or dwarfism is due to growth hormone deficiency.

3. A pharmaceutical composition for the promotion of tissue regeneration, which composition is characterized in that it comprises a therapeutically effective amount of inhibitor of dipeptidyl peptidase IV.

4. A composition according to any preceding claim, wherein the inhibitor of dipeptidyl peptidase IV comprises amino-acyl pyrrolidine nitrile.

5. A composition according to any preceding claim, wherein the composition is formulated for oral administration.

6. The composition according to Claim 5 formulated as a tablet or capsule.

7. The use of inhibitor of dipeptidyl peptidase IV in the preparation of a therapeutic composition for the treatment of sub-normal development or dwarfism or for the promotion of tissue regeneration.

8. A method for the treatment of sub-normal development or dwarfism, wherein the patient is administered a pharmaceutical composition comprising inhibitor ofdipeptidyl peptidase IV.

9. A method according to Claim 8, wherein the composition is administered once per day in the evening.

10. A method of tissue regeneration wherein the patient is administered a pharmaceutical composition comprising inhibitor of dipeptidyl peptidase IV.